REMARKS

Favorable reconsideration, reexamination, and allowance of the present patent application are respectfully requested in view of the foregoing amendments and the following remarks. The foregoing amendments have full support in the specification, at least, at pages 3 to 4. No new matter is entered.

Amendments

Claims 1 and 5 are amended. Claim 20 is added. Claims 7-19 are withdrawn.

Personal Interview

Applicant sand the undersigned wish to thank Examiners Hiriyanna, Woitach, and Kelly for the courteous and productive interview conducted on May 28, 2009. Because Applicant was not relieved of the duty under 37 C.F.R. § 1.33(b) of providing a summary of the arguments presented during that interview, Applicant provides the following comments. In the "substance of the interview" section of the Interview Summary, the Examiner states that "Applicant agrees…" in reference to the Examiner's argument that the instant claims only recite *in vitro* steps. Applicants did not expressly agree with the Examiner on this point, but did agree to consider submitting claim amendments that add more positive method steps which correlate the *in vivo* action.

Rejections under 35 U.S.C. § 102(b)

In the Office Action, beginning at page 3, Claims 1-4 were rejected under 35 U.S.C. § 102(b), as reciting subject matters that allegedly are anticipated by Amaar et al., and claims 1-5 were rejected under 35 U.S.C. §102(b) as being anticipated by Lai et al. (hereinafter "Lai"). Applicant respectfully requests reconsideration of this rejection.

Although Applicants do not necessarily agree with the Examiner's basis for the rejection, the claims now recite positive *in vivo* steps which correlate the *in vitro* action with an *in vivo* result. Such a correlation has never been shown before. Furthermore, neither Amaar nor Lai teach or suggest a screening method, but merely recognized the effect of Fhl2 overexpression. Since these references do not teach each and every aspect

of the invention, either inherently or explicitly, they cannot anticipate the claims.

In addition, both Amaar and Lai fail to show expression of Fhl2 in osteoblasts *in vivo,* which is now an explicit step of the claims. Amaar describes detection of expression of Fhl2 by Northern Blot (page 12056, Figure 3) and Western Blot (page 12057, Figure 4) in permanent cell lines. These cell lines are either permanent cell lines which were obtained from osteosarcomas of human origin (U-2 OS, MG-63, Saos) or primary cell lines which stem from the calvaria and the ribs and have been passaged three to four times (page 12054, right-hand column, third paragraph). Contrary thereto, the inventors show that expression of Fhl2 *in vivo* can be detected in osteoblasts in the bone of mice.

Although the claims are directed to the identification of a compound which can form an extracellular matrix in osteoblasts *in vivo*, it was clearly unknown in the prior art whether the *in vitro* effects can really be applied to an *in vivo* situation. Applicants have clearly shown that Fhl2 has an effect both *in vitro* and *in vivo*, and such a correlation is now an explicit positive step in the claims. Furthermore, the disclosures of these two citations cannot be anticipatory of the claimed invention because the detection *in vivo* verus *in vitro* is not just a mere technical difference. The inventors have shown that Fhl2 expression can be induced in all tissues examined by the inventors to date, as soon as the cells are taken into culture, irrespective of whether or not the tissue already expresses Fhl2 *in vivo*. Furthermore, Amaar only mention expression in human osteoblasts, but not *in vivo* expression of Fhl2 in osteoblasts. Thus, induction of Fhl2 expression in cell culture cells is an indirect and unspecific effect which does not allow any conclusion with regard to the role in bone formation.

Amaar present the hypothesis that IGFBP-5 may bind to Fhl2, a transcription modulator, to stimulate transcription of putative IGFBP-5 target genes that may be involved in regulation of osteoblast cell proliferation and differentiation (page 12059, right-hand column, last sentence of second paragraph). While this hypothesis is regarded as speculative by the authors themselves, it clearly refers to a role of Fhl2 on proliferation and differentiation of osteoblast precursors. It is therefore perfectly possible that there is a general effect of Fhl2 on the cell division rate and on the differentiation process as it has been described for Fhl2 in multiple cell culture systems from varying tissues. This

general *in vitro* effect of Fhl2 would inevitably have some effect on osteoblast precursors in the form of an indirect and unspecific effect on the mineralization and expression of osteocalcin after these cells have differentiated into mature osteoblasts.

The increase observed in proliferation and differentiation in cell culture observed by Lai after overexpression of Fhl2 is most likey an unspecific general effect found in many different cell culture systems using cells from various origins. Accordingly, one of ordinary skill in the art would not have expected that the effects observed by Lai to be specific or applicable to bone cells. Other groups have been unable to demonstrate these effects *in vivo*, which is supported by the finding that Fhl2 has no effect on proliferation or differentiation of osteoblasts *in vivo*. Furthermore, the unspecific effect in cell culture observed by Lai may also indirectly influence mineralization and expression of osteocalcin.

Contrary to these teachings, the inventors of the present application have shown a direct specific cell-autonomous and anabolic effect of Fhl2 on the activity of already differentiated osteoblasts *in vitro* and *in vivo*. In addition, the present inventors are able to present a molecular mechanism for this observed effect. It is also noteworthy that the present inventors could not detect any Fhl2 effect on proliferation of differentiation of osteoblasts or osteoblast precursors *in vivo* which contradicts the *in vitro* data of Amaar.

Specifically regarding the rejection over Lai et al., the group of Su-Li Cheng investigated the function of the co-factor FHL2 in the formation and maintenance of bone. The Lai reference cited by the Examiner is an abstract published in 2002 and only reports preliminary data of this work. The follow-up paper of this investigation was published in 2006, that is, after the filing date of the subject application. A copy of this later publication by the Cheng group is attached as Exhibit A.

The Lai abstract describes that FHL2 interacts with avβ5 Integrin. FHL2 localizes intracellularly at the focal adhesions and in the nucleus. Ectopic expression of FHL2 in the MC3T3-E1 cell line which exhibits characteristics of osteoblast precursors, led to increased cell adhesion, matrix mineralization and cell proliferation.

There is no reliable information, however, on the reason for these effects. Based on the data of the Lai abstract an accelerated differentiation of osteoblast precursors

and/or the enhanced proliferation would be the most obvious speculation. The preliminary observations are summarized at the end:

"FHL2 upregulates osteablast growth and differentiation and synergizes Cbfal and FGF2 activity. Thus, FHL2 may play an important role in bone formation."

In the later full publication Lai at al. could not confirm any of the postulated functions of FHL2 *in vivo* with untreated wild-type and knockout mice. Again they report that FHL2 stimulates osteoblast differentiation and proliferation.

The inventors of the subject application observed that female as well as male FHL2 deficient mice with a congenic C57BL/6 background showed a significant and remarkable loss in bone substance of about 30%. They could demonstrate that the observed osteopenia was alone due to a reduced osteoblast activity and not due to a differentiation of osteoblast precursors and also not due to a change in cell proliferation. This finding was confirmed in the inventor's later publication Gunther et al. (copy attached as Exhibit B). Accordingly, the inventors used for verification a cell line of already differentiated osteoblasts (7F2). In addition, investigation of ex vivo cultures of primary osteoblasts from wild type and FHL2 deficient mice confirmed their analysis that FHL2 *in vivo*, *ex vivo* and in 7F2 cells had only an anabolic effect on osteoblasts but not on their differentiation. This analysis is corroborated by the fact that the ectopic expression of FHL2 in osteoblasts in transgenic mice leads to an increase in bone cells due to enhanced anabolic activity. However, the inventors did not observe any change in osteoblast differentiation or proliferation in any of the systems investigated.

Lai et al. do mention an increased osteocalcin expression as a consequence of ectopic FHL2 expression. The observed change in osteocalcin expression, however, on the basis of the data of Lai et al., could also be an indirect effect which has nothing to do with FHL2. Contrary to the inventors' work, no functional relationship is described by Lai et al., let alone a molecular mechanism. The present inventors could demonstrate that FHL2 and RUNX2 form a complex on chromatinized osteocalcin promoter *in vivo* and that FHL2 acts as co-activator in dependence from RUNX2.

Remarkably, the group of Cheng could also four years after publication of the Lai et al. abstract not show any bone phenotype in FHL2 deficient mice (see Exhibit A). The analysis of the bone substance was made only with μCT. A detailed investigation in not decalcified bone slices was not conducted. Maybe these different observations were made because Lai et al. did not use congenic mice. However, a uniform genetic background (congenic mice) is crucial for investigations of the bone substance, as the bone substance varies up to more than 30% among different in-bred mouse strains.

In summary, despite their investigation of FHL2 deficient mice, *ex vivo* cultures from calvaria and ectopic expression of FHL2 in MC3T3-E1 cells, Lai at al. at no time described an anabolic function of FHL2 in osteoblasts.

For at least the foregoing reasons, Applicant respectfully submits that the subject matters of the Claims are not anticipated by Amaar et al. or Lai et al., are therefore not unpatentable under 35 U.S.C. § 102(b), and therefore respectfully requests withdrawal of the rejection thereof under 35 U.S.C. § 102(b).

Rejection under 35 U.S.C. § 103(a)

In the Office Action, beginning at page 4, Claims 1-6 were rejected under 35 U.S.C. § 103(a), as reciting subject matters that allegedly are obvious, and therefore allegedly unpatentable, over the disclosure of Lai et al. in view of the disclosure of Amaar et al. and Muller et al. Applicant respectfully requests reconsideration of this rejection.

The disclosures of Amaar and Lai have been fully discussed above. Furthermore, the primary reference of Lai et al. has been effectively argued in line with the above-presented evidence, and therefore, must be removed as effective prior art, either alone or in combination with any other reference. For the reasons presented above, there is no reason or motivation to combine the teachings of Lai et al. with any other reference and arrive at the claimed invention.

For at least the foregoing reasons, Applicant respectfully submits that the subject matters of Claims 1-6, each taken as a whole, would not have been obvious to one of ordinary skill in the art at the time of Applicant's invention, are therefore not unpatentable under 35 U.S.C. § 103(a), and therefore respectfully requests withdrawal of

the rejection thereof under 35 U.S.C. § 103(a).

Conclusion

For at least the foregoing reasons, Applicant respectfully submits that the present patent application is in condition for allowance. An early indication of the allowability of the present patent application is therefore respectfully solicited.

If Examiner Hiriyanna believes that a telephone conference with the undersigned would expedite passage of the present patent application to issue, he is invited to call on the number below.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and the Commissioner is hereby authorized to charge fees necessitated by this paper, and to credit all refunds and overpayments, to our Deposit Account 50-2821.

Respectfully submitted,

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